Studies on Avian Hæmoprotozoa: No. III.—Observations on the Development of Trypanosoma noctuæ (of the Little Owl) in Culex pipiens; with Remarks on the Other Parasites occurring.[1]

By

H. M. Woodcock, D.Sc.Lond.,

Assistant to the University Professor of Protozoology.

With Plates 29—31 and 1 Text-figure.

CONTENTS.

PREFACE.

I PROPOSE in the present paper to conclude the account of the work on the parasites of the Little Owl (Athene noctua) which I carried out at Rovigno during the spring and early

[1] For No. II of these Studies, "On the Trypanosome of the Little Owl," etc., vide 'Quart. Journ. Micr. Sci.,' vol. 57, 1911, p. 141.

summer of 1909. I have delayed publishing up to the present my observations on the developmental phases of the Trypanosome in the mosquito, as I hoped to be able, before now, to obtain the corresponding phases, and, indeed, the transmission back again to the bird, of Trypanosoma fringillinarum, here in England, to complete the work. This latter research, however, progresses, unfortunately, very slowly, so that I think it useful to publish my earlier observations without waiting longer, more especially as the only other worker who has written anything of late on this subject, namely, Mayer (**2**), in his account of the parasites of another owl (Syrnium aluco), has upheld the view that the Flagellates occurring in mosquitoes which have fed on an owl are developed from Halteridia present in the blood of the bird. In my "Notes on Sporozoa, No. IV" (**14**), dealing with the nuclear structure of Halteridium and Leucocytozoon, I think I have shown clearly that, considered from the standpoint of these parasites, everything is against such a connection of either with a Trypanosome. And the evidence which I have obtained from the Trypanosome side of the question is equally negative, and does not bear out Mayer's contention in the slightest degree.

General Account of the Experimental Work.

The time at my disposal for experimental work with Culex was only very short—about the last three weeks of June. Earlier than this the bred-out females would not take blood, and at the beginning of July I was unfortunately obliged to leave Rovigno. Five owls were used, to which it will be convenient to refer by their numbers, viz., 15, 16, 19, 22, and 23. The first two were quite free from any infection; No. 22 had only Leucocytozoon, while Nos. 19 and 23 both had Halteridium noctuæ, Leucocytozoon ziemanni, and Trypanosoma noctuæ. As regards Owl 19, the Trypanosome was for some reason or other excessively scanty, and was not, I believe, present at all in the general circulation (cf. Study No. II). The Halteridial infection of Owl 19 was quite typical, the parasites, which, of course, were in the usual form of male and female gametocytes, being fairly numerous, and many of them full-grown and ripe for liberation from the host-cells. On the other hand, the Halteridial infection of No. 23 was not typical. In this owl nearly every red blood-cell was infected, usually with several (four or more) small forms, which in many cases, as they had increased in size, had united together into a kind of common plasmodium. This condition has been already described by me elsewhere (**13**). I observed hardly any full-grown, normal gametocytes in the blood of this bird. Owls 22 and 23 only arrived on June 12th.

Early in June, finding that my bred-out females would not yet bite, I left Owl 19 one evening in a cage into which I had put half-a-dozen or so "wild" females, caught in the same little outhouse, used as a "dark-room," where Schaudinn himself had caught many in time past. Before midnight two of these had fed. I may note here that the favourite place for feeding of the mosquitoes was the fleshy pad just above the bird's nostrils. One of the two females was examined after about thirty-six hours had elapsed, this being the period when, according to Schaudinn, the ookinetes become transformed into Trypanosomes. Its stomach contained a number of fully-developed ookinetes, i. e. which had lost all the pigment. The majority had the characteristic curved form, but did not appear at all active. A few, however, showed a certain amount of activity, which consisted in tending to straighten out, and again recurve, the body, either slowly or now and again spasmodically. I never observed any marked forward progression of the ookinetes.

Besides the ookinetes certain other bodies were found to occur, very scantily, in the preparation. These elements were more elongated and spindle-shaped, somewhat resembling an Indian club in form, one extremity tapering finely, the other being rounded off. Further, some of them were very slightly curved or crescentic. Apart from the difference in shape, the general appearance of these bodies, observed living, was not at all unlike that of the ookinetes. They were quite non-motile. While I was in the act of observing one, and wondering whether it was a later stage in the development of an ookinete, it gave one or two very slight, jerky movements, and before I had fully assured myself that these really represented active, voluntary motion on the part of the parasite, to my great surprise it had developed a flagellum. I thought I had just an indication of the tapering end of the body beginning to lengthen, but more than this I did not see. One moment the parasite had no flagellum, an instant later it had a fully-developed free flagellum, about three-quarters as long as its own body. The process must have been exactly comparable, in short, to that which I have since found to occur in Leptomonas ("Crithidia") fasciculata (vide **15**). I hurriedly brought a colleague, Dr. Reichenow, then also working at Rovigno, to look, and he, in turn, saw the process repeated in the case of another individual. These were the only two instances in which we saw the development of the flagellum, and we only found one other flagellated form in the preparation. We both carefully examined several of the other (non-motile) elements, and satisfied ourselves that they had no sign whatever of a flagellum. If I may be permitted the personal reminiscence, I well remember how, in the excitement of the moment, we were both of us firmly persuaded that we had seen the most important stage in the transformation from an ookinete into a Trypanosome, as it had been described by Schaudinn. This confidence did not endure, however, for many days. I continued to watch certain ookinetes during the afternoon, and felt very disappointed that I could not see any indication whatever of a typical ookinete passing into one of the fusiform bodies. After keeping the preparation under observation for about three hours I removed the coverslip and made smears, which were fixed and stained.

Most unfortunately, Owl 19 was taken ill and died on the following day, and for some days I had no owl infected with

Trypanosomes. During this interval I fed several caught mosquitoes on an uninfected bird (either 15 or 16), and these were examined at periods of from thirty-six to fifty-eight hours after being fed. In two cases, elongated, fusiform elements, perfectly similar to those above-mentioned, were found; but none of them was seen to become active or develop a flagellum. In another female, examined about fifty-four hours after being fed, numerous active Flagellates were observed in the stomach; these differed considerably in appearance from the "resting Flagellates," but, on the other hand, agreed closely with the characteristic developmental forms described below, and I have not the least doubt that they also belonged to the life-cycle of some Avian Trypanosome. It is important to note that no ookinetes were seen in any of these mosquitoes. These observations showed not only that the fusiform resting Flagellates might probably have another origin than from the ookinetes of Halteridium, but also that it was essential to use only bred-out females, in order to follow the course of development taken by the blood-parasites in the Culex; hence, from this time onwards only such individuals were used.

On June 12th Owl 23 arrived, and during the night of the 15th–16th I found Trypanosomes in the peripheral circulation; the parasites were of the characteristic fusiform, rather stout type (Pl. 31, fig. A), described by Minchin and myself (l. c.). The Trypanosomes were not at all infrequent—for Avian Trypanosomes, it must be remembered—in the peripheral blood at this time,[1] and were found also on other occasions. In both of the two first mosquitoes which were examined after being fed on this bird, after intervals of about thirty-four and forty hours respectively, numerous active Flagellates were found in the stomach. Digestion was proceeding normally and was about half accomplished, or rather more. (I may state here that females which had taken blood were always kept at a tem-

[1] In any living preparation, consisting of a small drop of blood spread out into a thin layer under a coverslip seven-eighths of an inch square, there would be one or two Trypanosomes.

perature of from 25°–27° C., at which temperature digestion took three to four days to be completed.) The development of the Trypanosomes appeared to be at about the same stage in both the females, and in both similar phases were observed.

Ookinetes of Halteridium were also found, but in both mosquitoes they were few in number—quite scarce, in fact, when compared with the number present in the first female examined, which had fed on Owl 19 (cf. above). This was readily to be understood, bearing in mind the different condition of the Halteridial infection in the two birds; in spite of the very strong infection of Owl 23, there were nothing like so many full-grown, ripe gametocytes as in Owl 19. A living preparation made from one of these two stomachs was kept under observation for some time. Three ookinetes in different fields, all of which had lost their pigment grains, were noted at intervals during two hours, but none of them showed any change in form or the slightest indication of any development into one of the fusiform bodies or into a flagellate condition. The preparation was again looked at two hours later, with the same result in the case of two of the ookinetes; the third could not be found. By this time most of the Flagellates seemed to be dead—at all events, only two or three could be observed, and these were very sluggish.

Altogether, twenty-six female Culex pipiens which had fed on Owl 23 were examined, after intervals varying from about twelve to eighty hours. Flagellates were found in twelve of these, i. e. in about 46 per cent.; sometimes they were numerous, in other cases only few were seen. On the other hand, out of thirty-two females fed on one of the other owls (Nos. 15, 16, 22), none of which was infected with Trypanosomes, the stomachs of which were carefully examined after different intervals (thirty-six to fifty-four hours), in not a single case were the parasites found! Two of these birds were quite free from any Hæmoprotozoan infection; Owl 22, however, had a fairly strong infection of Leucocytozoon ziemanni. Seven of the thirty-two mosquitoes examined had fed on this latter bird,[1] and in four of them a few of the large ookinetes of Leucocytozoon were found. In form and appearance these were very similar to the ookinetes of Halteridium, but they were considerably larger. Those I observed were quite motionless, and did not change at all in shape.

The fifty-eight mosquitoes examined were barely half the total number (about 120) which fed on blood. The mortality amongst these newly bred-out imagines was high, and it appeared to make little difference whether they took blood or the sugar-water, banana and prune juice with which they were supplied. More than a quarter of those which fed on blood died during the course of digestion. During the short time at my disposal I had, therefore, only very limited material for transmission-experiments. During the last fortnight between fifteen and twenty females which had fed on Owl 23 and successfully completed digestion, were given the opportunity of feeding on an uninfected bird (either 15 or 16). To my very great disappointment, however, not one of these could be induced to feed again. Some of them drank a little water, or partook of the food-supply which was placed in the same cage for a few hours during the day-time for the males to feed upon; nevertheless, many of them gradually died off during this period. I was loath to sacrifice any more for examination, hoping to the very morning of my departure from Rovigno that one or more would bite again and give me the chance of seeing whether one of the uninfected birds would become infected. Unfortunately, the endeavour did not succeed. Owls 15 and 16 accompanied me back to England and lived for many months—much longer than a single bird did at Rovigno—but neither of them ever showed any parasites at all.

While restricting myself to bred-out females for the experimental work, incidentally I examined a few more caught "wild" mosquitoes. None of them was infected with active Flagellates (i. e. Trypanosome developmental forms), but again

[1] This owl died on June 22nd, so that I had it only ten days.

two or three contained the peculiar, spindle-like resting forms, above noted; in no further instance, however, did I see one develop a flagellum. I have since wished that I had been able to devote more time to the study of this parasite, and to ascertain, for example, whether it occurred in male mosquitoes also. But I was intent on proving the origin of the Flagellates which developed in the blood-fed females, and in transmitting them back to the birds, if possible, and this took every minute of my time, as I had no assistance whatever and had everything to do myself.

Description of the Parasites, as they were found in the Mosquitoes.

(i). Trypanosoma noctuae.

I pass on now to describe the developmental phases of the Avian parasites in the female Culex pipiens, and begin with those of Trypanosome noctunae. Considering the Flagellates, first of all, as they were seen in life, in mosquitoes examined about thirty-six hours after being fed on an infected owl, the most striking form, which at once held my attention on first seeing it, was a very long, extremely slender type, which progressed rapidly, by means of its flagellum and the undulating membrane along the anterior part of the body. The membrane appeared to be very narrow along the middle region of the body, and on account of this fact and the active movements of these individuals, I could not determine exactly where it ended, or whether this type was trypanomonad (crithidial), or trypaniform. In fixed and stained preparations, however, it is seen to be distinctly and invariably trypaniform (figs. 13–21). Nevertheless, these attenuated trypanosomes were entirely different in appearance from an ordinary, slender, elongated blood-form of Trypanosome, e. g. a piscine type such as T. granulosum. While the anterior part of the body, where the membrane was conspicuous, was sinuous and flexible, the hinder part, as a rule, nearly half the entire length or more, was held quite stiffly and did not appear to be actively flexible at all. Frequently it was practically straight, but in some individuals it was quite curved round, like a crook (cf. figs. 19–21); this posterior part would retain this shape, unaltered, even while the parasite was moving rapidly forwards. From a comparison of stained preparations it is evident that this portion of the body represents a prolonged extension of the cytoplasm behind (posterior to) the kinetonucleus. In view of my observations on the living parasites, I regard this cytoplasmic "tail" as differing from the anterior half of the body in lacking anything of the nature of myonemes, and consequently any ability to bend or twist of itself. I consider that it is only, as it were, passively flexible, and that any curving or bending is produced mechanically, as the result of contact with the blood-cells and other elements among which the parasite happens to be working its way. What function this remarkable development serves, I was not able to ascertain.

The above type of individual constitutes a fair proportion of the total number of flagellates present, even at this somewhat early stage of the development. The other types of parasite seen were for the most part relatively short trypanomonad (crithidial) forms and individuals representing every possible transition between such and the extremely attenuated forms. As is shown by the fixed and stained preparations made of stomachs at about this period, the majority of these intermediate forms are really trypaniform, i.e., the kinetonucleus is on the aflagellar side of (posterior to) the trophonucleus. The shorter, more typically trypanomonad individuals resembled the commonly occurring crithidial forms which develop in cultures of an Avian trypanosome, such as I have described in the case of T. fringillinarum. All these forms had what is usually distinguished as the crithidial type of movement. Progression was in a slightly zig-zag manner, the flagellum and the anterior part of the body (corresponding to the position of the undulating membrane) vibrating actively. The movement of these forms was not nearly as rapid as that of the long, slender individuals. Lastly, other forms noted were short and pyriform, and moved jerkily, not displacing themselves to any extent; these were infrequent. Notwithstanding the great increase in number of the parasites which must have taken place since the blood entered the stomach, I found scarcely any individuals actually dividing. In one or two cases I saw trypanomonad individuals with two flagella, and in one instance a pair of such forms still connected together; in another instance which I noted, division was markedly unequal, a short, pyriform individual becoming separated off from a distinctly larger, broader, club-shaped form.

At a later period of the development the long, attenuated forms predominate more and more, until in females examined fifty-five hours or subsequently after being fed, the stomach contained apparently only such forms; and this is borne out by the study of fixed and stained preparations made of stomachs of this period or later.

All my observations on the parasites relate to the stomach-phases of the life-cycle which occur between thirty-two and about seventy-six hours after the mosquito has fed. I examined four females from twelve to eighteen hours after feeding on Owl 23, but I could not find any Trypanosomes at this early period. It would be necessary to examine many individuals to find the earliest changes in the parasites, because, even if the development is proceeding all right, the Trypanosomes have not yet had time to multiply and give rise to any considerable number of parasites; and it must be remembered that only very few Trypanosomes are taken up by the mosquito from the blood to start with. Neither did I find any phases of the parasites in the intestine; but this did not surprise me, because nearly all the mosquitoes were examined, at any rate, some time before the stomach was quite empty. While digestion is still going on, the stomach is most certainly the principal, if not the only situation in which the Trypanosomes occur. If I had been able to examine a sufficient number of females after digestion had been completed, or after they had had another meal of blood, the parasites might have been found in the intestine; I shall discuss this point later on. My observations are, I am aware, only incomplete; but they were the fullest I was able to make in the circumstances, and bearing in mind the chief objects on which I concentrated my attention during the short time at my disposal, namely, to determine whether the Trypanosome-phases in the mosquito were derived from the Halteridial parasites or not, and to bring about, if possible, the transmission of the Trypanosomes back again to the owl.

The Trypanosomes in Permanent Preparations; Comparison with the Developmental Stages found in Cultures.—I think, however, by comparing the various forms which I did obtain in the mosquito, with the development which I have found to take place in cultures, in the case of another Avian Trypanosome, T. fringillinarum, that a general idea can be arrived at of the main course of the natural development in the stomach of the insectan host; as already indicated, the chief gap and element of uncertainty relates to the earliest changes undergone by the parasites.

I have no hesitation in making use of the cultural development for this purpose, because practically all the different forms occur in both cases. The reason it is helpful is because, in the culture, the development continues over a much longer period owing to the fact that there is no absorption of the medium, such as occurs in the insect's stomach, and therefore the intermediate forms are met with abundantly and stages in division are frequent, the latter being of much assistance in determining the sequence of the developmental changes. In the Culex, on the other hand, the course of digestion is comparatively rapid and completed in three to three and a-half days, by which time the stomach is empty of blood. It is undoubtedly in relation with this fact that we find the early and intermediate stages in the development of the parasites passed through very quickly, which leads on to the production of the ultimate stages found in the stomach. This development proceeds along two lines, the result being the formation of two extreme types. The great difference in the relative frequency of certain forms, on the one hand in the culture, and on the other in the mosquito's stomach, is entirely in accordance with the different conditions prevailing in the medium in the two cases.

The earliest developmental forms obtained are trypanomonad individuals, such as are drawn in figs. 1–3, or *b* and *c* of the scheme. These are medium-sized, fusiform parasites, with a distinct membrane and the two nuclei close together, the kinetonucleus being usually just posterior to the middle of the trophonucleus. Both nuclei are either about the middle of the body or else slightly in the hinder part. This type of individual corresponds exactly with a particular crithidial form which occurs commonly in cultures, such as is drawn in figs. *f′–j′* of the parallel series of stages from cultures, which I have reproduced [1] for ready comparison with the developmental forms in the mosquito. In the mosquito, however, even in my earliest preparations (about thirty-two hours), the number of those trypanomonad individuals is relatively very small. This is in marked contrast to what is the case in the cultures, where the trypanomonad type is by far the predominating one. In the cultures many of these forms are distinctly larger than those with which I compare the mosquito forms just described (cf. figs. *b′–d′*), and have the undulating membrane often better developed. In a small proportion of them, moreover, the kinetonucleus is just in front of the trophonucleus, instead of being alongside, a condition which I have not observed in this phase in the mosquito.

As regards the immediate origin of these trypanomonad forms in the stomach, I think it is most probable that they arise by the division of rather larger, but otherwise similar forms. As is seen very clearly from the full series of the crithidial forms figured in my memoir (l. c., Pls. 27, 29), the

[1] All these figures of cultural forms are taken from my Memoir in the ' Quart. Journ. Micr. Sci.,' vol. 55, 1910, pl. 27, 29, or 30.

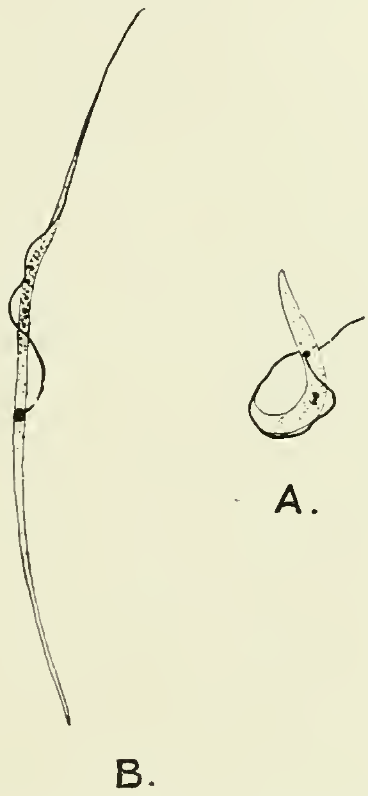
division of the larger (earlier) individuals is at first by practically equal binary fission, the only inequality being that the daughter-flagellum may be shorter than the parent one (cf. figs. *e′–g′* of the scheme, Pl. 31). Unfortunately I have not found any typical trypanomonad individuals in the act of the dividing in my preparations, but, as already mentioned, I observed an instance in life in which the parasite was just completing division, and in a condition practically identical with that seen in fig. *g′*.

However, I have very little doubt that the division of these trypanomonad individuals with the nuclei about the middle of the body is almost, if not quite, finished by the time of my earliest preparations. Because there are very few forms still left in this phase of the development; most of the parasites occurring are individuals which have lost the trypanomonad condition already and become trypaniform, and are either at some stage in the development of the elongated, attenuated trypanosome-phase, or have, in fact, attained the latter. I have been able to find, in stomachs of from thirty-two to thirty-six hours, a regular series of transitional forms in this connection (cf. Pl. 29, figs. 4–10). The elongated trypaniform condition is reached by a progressive modification of the form of the above trypanomonad individuals. I think this change is most probably unaccompanied by further division, at any rate, to any extent; otherwise, I ought to have found some indication of the process, as the parasites are fairly numerous, and all degrees in the gradual change of the type of form are to be met with.

The earliest change is the passage of the kinetonucleus, and, of course, of the blepharoplast and attached flagellum, definitely behind the trophonucleus, the latter retaining its position (figs. 4 and 5). Next, the general cytoplasm in the posterior half begins to increase considerably in length; in this way the parasite becomes longer, but it does not increase much in breadth. On the contrary, as the length increases, the body becomes ultimately not only relatively, but actually narrower and more slender. The change is most probably the result of two factors combined, namely, growth to some extent, and a thinning-out of the general cytoplasm. The latter factor is evident from the change in the form of the trophonucleus. This body, in the early stage, has the shape of a short and broad oval (figs. 4–6); as the trypaniform condition develops it becomes elongated, and appears as a narrow oval, compressed laterally (figs. 7–10). Meanwhile the kinetonucleus has passed well back behind the trophonucleus, but it never approaches anywhere near the posterior end of the body, the extension of the cytoplasm backwards, as the "tail" develops, being so great (figs. 8–12). The final position of the kinetonucleus is usually about the middle of the body, or slightly in the posterior half. Nevertheless, the attached flagellum and the membrane are relatively long; the membrane is always narrow and rarely at all wavy. Although, as the parasite assumes its final attenuated condition, the anterior part of the body also becomes thinned out and tapering, it does not appear to increase in length to anything like the extent that the posterior half does; so that the trophonucleus lies, as a rule, distinctly in the anterior part of the body, at times comparatively near to the anterior end (cf. figs. 13–20).

As the final stage in the development of this form is nearly attained, a characteristic change usually occurs in the appearance of the trophonucleus. This organella, which has already become narrowed and compressed, becomes broken up into many oblong or rather rod-like blocks, arranged with their length transversely to the long axis of the body of the parasite, thus giving the whole nucleus a remarkable ladder-like appearance (figs. 13–17). Apparently this change represents really the break-up of the original large karyosome, which is not seen, as a rule, in the nucleus when stained by Giemsa, into several small karyosomes arranged in a row. This is shown by the comparison of wet-fixed preparations stained by iron-hæmatoxylin, in which slender elongated trypaniform individuals, approaching the final form, have, instead of one large karyosome (as in Text-fig. 1 *a*), four or more small karyosomatic bodies (Text-fig. 1*b*). Probably each rod or block in the Giemsa-stained parasites corresponds to a karyosomatic grain of the greatly extended trophonucleus. Whether this change in the nucleus is absolutely necessary before the final form can be said to be completely

TEXT-FIG. 1.



× 1600. Stages of *Trypanosoma noctuæ* in the mosquito, showing different conditions of the trophonucleus (from wet-fixed film, stained by iron-hæmatoxylin). A. Intermediate trypaniform phase. The karyosome of the trophonucleus is apparently budding off a small daughter karyasome. B. Approximately final (propagative) form. There are five distinct karyosomes forming the elongated trophonucleus.

formed, I do not feel certain; occasionally individuals, which in other respects seem to have reached the final stage, are found where the nucleus is not ladder-like (cf. figs. 19–21).

In my preparations of stomachs of fifty-five hours or later, scarcely any intermediate changes are present, practically all the individuals having attained the attenuated trypaniform condition. These are certainly very striking in appearance; their thread-like form can be judged from the fact that their length is generally from 52 to 56 μ, while they are rarely more than 1 μ broad at the widest part, and often scarcely that! Superficially, they almost deserve the name of spirochætiform Trypanosomes, were that not so very misleading. The half of the body in front of the kinetonucleus—the actively motile part—is frequently spirally twisted or coiled to some extent; while the long, passive cytoplasmic "tail" behind the kinetonucleus may be either practically straight (figs. 13, 14, 18 and 20) or bent up on itself in a curve (figs. 15–17, 19 and 21).

The above-described trypaniform type also occurs in the cultural development. I have observed individuals both in the intermediate condition, and in what undoubtedly corresponds to the ultimate form attained in the mosquito's stomach (cf. figs. *k′–m′*). It is important to note that the proportion of such forms to the trypanomonad individuals in the cultures is just the reverse from what is the case in the mosquito after thirty-two hours. This provides a very interesting commentary on the course of the cultural development as compared with that of the natural one. Whereas, in the latter, the enviromental conditions favour and lead on to the production of the attentuated trypaniform type, by the modification of the ordinary trypanomonad individuals, in the cultures there is not the same stimulus (the approaching completion of digestion) to the production of this form, and in consequence it is only developed, as it were, in isolated instances; the multiplicative ("crithidial") form, on the other hand, persists and increases in numbers, far beyond what occurs under the natural conditions. The intermediate forms in the cultures resemble closely the corresponding individuals from the insect's stomach (cf. figs. *e–g*). The few final forms which I have found (fig. *m′*) also agree quite obviously in their chief features; there is the same long, cytoplasmic "tail," and even the ladder-like nucleus may be developed. The principal difference is that the individuals in the cultures are much broader relatively than the natural "spirochætiform" individuals; their body has apparently more bulk. I think this is simply an indication that these forms have grown more in the cultural medium than they do in the stomach; and division having most probably ceased, the result is the marked increase in "girth."

To consider next the other line of development followed by the parasites in the mosquito, this proceeds also from the original type of trypanomonad individual, by a modification in form and the mode of division. Here, again, I think the course of events can be understood from the cultural development. In certain of the ordinary trypanomonad individuals, the nuclei show a tendency to be in the hinder part of the body. This may be in the first place due to a slight obliqueness in the direction in which the preceding nuclear division has occurred. At any rate, in such individuals the direction of the nuclear division is usually oblique, and we find not only the persistence of the nuclei in the hinder part of the body, but a distinct tendency already for the division of the body to be slightly unequal (cf. fig. *n′*). These individuals with the nuclei definitely in the posterior half of the body pass, or grow into the characteristic club-shaped forms,[1] the general body-protoplasm tending to be concentrated in the region of the nuclei (fig. *o′*). Now, in these forms, the division is always markedly unequal. It is important, I think, to note this particular point, that where the nuclei are situated about the middle of the body, the division is practically equal (as in the ordinary trypanomonad individuals); where, on the other hand, the nuclei are distinctly in the hinder part of a club-shaped individual, the division of the cytoplasm is unequal. This is seen in figs. *p′* and *q′* of my scheme and also in several other figures in my memoir (**12**). I consider this mode of division is primarily due to the drawn-back position of the nuclei, and consequently of the blepharoplasts, and to the fact that the new daughter-flagellum is, at any rate, largely

[1] I formerly distinguished these by the cumbrous term of "accentuated trypanomonad" individuals.

formed by free, independent growth. Individuals which are manifestly the product of a similar division are seen in figs. *r* and *t′*.

Now, what I have found in the mosquito agrees entirely with the above description. One of the earliest stages along this line of development is the characteristic club-shaped trypanomonad form of fig. 22, or *k*. This individual corresponds very closely, it will be seen, to that of fig. *o′* or *p′*; it is almost ready for division. As remarked in my account of the living observations, I saw one instance of such a form in the act of unequal division. The smaller of the daughter-individuals about to result was a short, pear-shaped form, with scarcely any membrane; the larger one was a rather club-shaped individual, long and tapering, and probably with a long, narrow membrane (cf. fig. *q′*). Unfortunately, I have not obtained any examples of individuals just dividing in my permanent preparations; in view, however, both of the living observation and of the fact that numerous individuals belonging to both the distinct forms which are produced by such division occur in my preparations (cf. figs. 24, 26, and again, figs. 23, 25), I cannot doubt that such unequal division takes place as a normal and typical phase of the development in the mosquito.

By further division of the larger daughter-individual (possessing the long membrane), smaller forms are produced (figs. 30–32 or *n* and *o*); these can at first be recognised as corresponding to one or the other of the two types, but in the smaller individuals the distinction between the daughter-forms tends to be less marked. Here and there I have found a stout pyriform individual, one of the early-developed pear-shaped daughter-forms, just about to divide, after further growth (fig. 27); and also a quite small parasite, one of the ultimate stages, I should say, undergoing division (fig. 34). There can be no doubt that this line of development leads ultimately to the production of small, pear-shaped or oval parasites, with the nuclei close together and situated about the middle of the body, or nearer the posterior end, and with the flagellum drawn back but with practically no membrane (figs. 29, 32 and 33). Here we have, unmistakably, the haptomonad phase of the Trypanosome, as I have proposed (**16**) to term the so-called "gregariniform" phase, which serves for attachment (and coincident multiplication). If these figures, for instance, are compared with certain of the text-figures in my description (**15**) of Leptomonas fasciculata, as I found this parasite in Culex pipiens, it will be perfectly clear that they represent the corresponding phase of the Flagellates in both cases. This agreement, I may incidentally mention, affords an excellent example in favour of the point I have urged, that from the haptomonad phase alone (having regard only to the morphology) it cannot be said with certainty whether a particular Insectan parasite represents a Leptomonad, a Crithidia or a Trypanosome.

From the above account it is clear that the early development of Trypanosome noctuæ in the mosquito culminates in the production of two distinct and extreme types. Whether the above description includes all the modifications of form which occur in the life-cycle in the Insectan host, I am not able to say. I think, however, that it is not difficult to interpret the significance of the end-stages, and if the view I favour is correct, any further stages in the life-history represent in the main a repetition, or re-development of the above sequence of forms, consequent on the persistence of the parasites in the mosquito and their response to fresh meals of blood.

The haptomonad forms most probably—as, indeed, is implied by thus designating them—become attached to some part of the wall of the alimentary canal, lose practically all the flagellum and enter upon the resting phase; "resting," that is to say, as regards locomotion, but not in regard to nutrition or multiplication. I think there is no reason to doubt that the chief situation favoured by these haptomonads for attachment, as the digestion becomes finished, is the anterior end of the stomach and especially the invaginated epithelium of the proventriculus, as was so graphically described by Schaudinn (**10**) and illustrated by him in Text-fig. 14. Just because the parasites were in this situation, I feel sure that in regard to this point Schaudinn's account relates to actual developmental phases of T. noctuæ and not to a purely Insectan form (such as, possibly, Leptomonas fasciculata). In the case of the latter, on the other hand, the haptomonad forms are restricted to the intestine apparently. Whether the haptomonad phase of T. noctuæ also invades the intestine to any extent, I am unable to say; considering that these forms tend to mass themselves at the anterior end of the stomach, it is quite likely that they do not—unless they are also able to form cysts for passage to the exterior.

Returning now to the first line of development, what is the further destiny of the remarkable thread-like individuals? I am decidedly of the opinion that they represent the propagative phase of the Trypanosome in the mosquito, the form, that is, in which the parasite is finally transmitted back again by inoculation to the owl. Unfortunately, I have no proof of this, but certain considerations point strongly to this being the right explanation of the significance of the above very characteristic type. In the first place, a brief comparison with the known course of the life-history in piscine Trypanosomes is most suggestive. It has been recognised for some time that a given species of fish Trypanosome shows—at all events, in many cases—very considerable polymorphism during that part of the cycle undergone in the blood of the Vertebrate host; thus certain individuals of a species may be quite small, slender forms (representing a young stage), others large, massive forms, with all intermediate grades between cf. T. granulosum, T. percæ, Minchin [**3**]). Now I have shown that the same pronounced polymorphism also obtains in Avian Trypanosomes (vide [**12**] and, with Minchin [**5**]). Whereas earlier writers frequently described a small form from a particular host as one species, and a large, massive form from the same host as another species, the true meaning of these different types is that they are different stages in the life-history of one species. Thus the large, massive, "blue" Trypanosomes of the little owl, originally described under the name T. ziemanni, and connected by Schaudinn with Leucocytozoon ziemanni, are really only the large forms of T. noctuæ and not a separate species at all. And a corresponding great variation in form and size is found in T. fringillinarum, in the chaffinch. Hence there is a strong family resemblance between piscine and avian Trypanosomes, as regards the types met with in the Vertebrate host; and it seems quite clear that both belong to the same group or division of Trypanosomes, and stand somewhat apart from Mammalian forms, for instance. Turning now to the life-cycle of piscine forms in leeches, which has been fully worked out by Miss Robertson in two or three cases (e.g. T. raiæ in Pontobdella [7 and 8], T. danilewskyi in Hemiclepsis [9], it is found that, after a varying period of multiplication by the parasites in the trypanomonad (crithidial) phase, as the digestion approaches completion—the time occupied varying considerably according to the conditions—a trypaniform type is developed, which becomes very elongated and slender. This passes forwards from the crop into the proboscis-sheath, and is the propagative, inoculative form, which transmits the infection to a fresh fish.

This type of form is essentially similar to that above described in Culex, the significance of which I believe to be also the same; in the leech it does not apparently attain the same degree of tenuity and the remarkable thread-like appearance which it does in the mosquito, but it may at times show the same peculiar ladder-like nucleus. It may at first be thought that from thirty-four hours onwards is too soon for the final, propagative form in the mosquito to be already present. But this rapid development appears quite explicable when the habits of the mosquito are considered. Unlike most of the Invertebrate hosts (transmissive agents) of Trypanosomes, mosquitoes do not feed solely on blood. On the contrary, it is generally recognised that, so far as the species of temperate climates are concerned, meals of blood are not by any means the rule, and in many cases only taken in connection with the development of the eggs. In the case of most females blood appears to be necessary for this purpose; it certainly is so in *Culex pipiens*, and as I have recently shown (l. c.), two meals of blood will suffice to bring about the growth and oviposition of the fertilised ova. After laying one, or perhaps two, batches of eggs, most of the females either die or go into hibernation. Hence, it is perfectly clear that unless the propagative phase of the Trypanosome is developed in time for inoculation at the second (or at most the third) meal of blood, the chances of the parasite passing back to the bird are very uncertain—an entirely different state of affairs from what is the case among tsetses or leeches. For the above reasons, therefore, I think there is no difficulty in assuming that the thread-like "spirochætiform" individuals which I have described represent the inoculative type, or in understanding their early development in the mosquito.

While I consider it is quite likely that these forms are inoculated again into the blood at the second time of feeding, I do not overlook the possibility that they (or some of them) pass first into some other organ of the mosquito (such as the salivary glands, or œsophageal diverticula), and there await a third meal; but this has still to be ascertained. I do think, however, Schaudinn was mistaken in stating that the Trypanosomes cannot be transmitted back again to the owl until the fourth meal inclusive. I should say, if the parasites had to wait until the mosquito took a fourth meal of blood, they would rarely, if ever, have the opportunity of getting back again at all, unless, perhaps, they were in a female which was going to hibernate. It is obvious from Schaudinn's account that he was following chiefly the multiplication of the parasites in the haptomonad condition (*vide* his description of their attachment in vast numbers to the walls of the alimentary canal). Schaudinn does not appear to have seen the real propagative phase at all! He nowhere describes the attenuated forms with the characteristic cytoplasmic "tail" (cf. on the other hand, Mayer's account, referred to below). Schandinn's slender, "spirochætiform" individuals, which he describes under "Spirochæta" (Trypanosoma ziemanni), agree fairly well with the intermediate trypaniform stages in the development of the final type (such as, e.g. figs. 7 and 8); but I must add, nothing has astonished me more than the lack of close correspondence, in the main, between the different forms of the Trypanosome occurring in the mosquito, as Schaudinn figured them, and as I have found them.

II. The Ookinetes of Halteridium noctuæ and Leucocytozoon ziemanni.

A few words next concerning the ookinetes of Halteridium and Leucocytozoon, as they occur in my permanent preparations. As regards their general appearance, I have little to add to the description previously given by Mayer (l. c.). The ookinetes of both forms are fundamentally similar in type, as was of course to be expected, considering that two essentially similar parasites are concerned. In both cases the body is very frequently more or less coiled up in the form of a C. Practically the only difference between the two is that the ookinetes of Leucocytozoon are much larger, especially in regard to length, than are those of Halteridium (c. f. figs. 35–40 and 41 and 42).

In my preparations of the Culex which fed on Owl 19, made from thirty-three to thirty-six hours after feeding, the ookinetes of Halteridium are very numerous. In preparations made from mosquitoes which fed on Owl 23, ookinetes are also usually to be found (up to thirty-six hours), but they are very scanty; this difference is due to the different conditions of the infection in the two owls respectively (see above, pp. 400–401). Speaking generally, all the ookinetes observed are in the same phase of development, and with one or two exceptions have lost all the pigment. In none of them is anything like a kinetonucleus, let alone a developing flagellum, recognisable. In a certain number a small chromatinic area, or clump of chromatinic grains, is present, in addition to the nucleus (figs. 35 and 36); but these stain quite similarly to the nucleus, and in no case have the characteristic appearance of a kinetonucleus, as seen when stained by Giemsa (contrast the "resting flagellates" referred to below). Unfortunately I am unable to say whether the nucleus possesses a central karyosome or not, but at any rate there is certainly no excentric or extranuclear karyosome, such as occurs at certain periods both in Halteridium and Leucocytozoon when in the blood,[1] and which often simulates a kinetonucleus in so marked a manner that it was formerly mistaken for one, until I showed clearly (**14**) what its true significance was. Frequently the ookinete shows one or two vacuoles in the cytoplasm; I have not been able to find any ookinetes in my preparations of mosquitoes which were made later than fifty-four hours after feeding.

I have only come across very few ookinetes of Leucocytozoon on my smears; I was generally able to find one or two during the living examination of the stomachs of mosquitoes which had fed on an owl infected with this parasite, but unfortunately in several cases, owing to their scantiness, I have not succeeded in obtaining any on the permanent slides made. Those I have found are all practically similar in form and size (figs. 41 and 42). The nucleus is always situated fairly near to the more rounded end of the body, as it is also in Halteridium; it varies in size to some

[1] Reichenow, in a note on Leucocytozoon ziemanni in his account of the Hæmogregarines in 'Handb. d. Path. Protozoen' (**6**) states that he was unable to find a karyosome in the male gametocytes. As I showed in my "Notes on Sporozoa," published about the same time, there is certainly such an organella present, excentric or even extranuclear as in the female forms. It is unmistakable in preparations stained by iron hæmatoxylin, but it is rarely shown in Giemsa smears. Reichenow does not say whether the figure he gives is from a Giemsa smear or not, but from its general appearance (e. g. the hypertrophied host-cell nucleus stains quite differently after iron-hæmatoxylin), I should say it was.

extent. Now and again separate chromatinic granules occur close to it (cf. fig. 42). In these ookinetes, also, one or two vacuoles are often present. None of the ookinetes of Leucocytozoon, any more than those of Halteridium, are in any later stage of development. I have not seen the least indication of the remarkable skein-like formation so graphically described by Schaudinn, accompanied by nuclear multiplication and the eventual development of a number of very small Trypanosomes! I must say that I doubt very much now the correctness of all this.

III. The "Resting Flagellates."

As was described in the account of the living observations (see above, pp. 401–402), in a small number of female Culex pipiens caught wild, which were examined at an early stage of the experimental work before I restricted myself to the use of bred-out mosquitoes, certain characteristic and rather peculiar motionless bodies were sometimes present. I have mentioned how on one occasion such resting forms were actually seen to develop into active flagellates, and the temporary illusion fostered by the observation. But not only were they seen in the first "wild female," which fed on Owl 19, well infected with Halteridia, they also occurred now and again in females which had fed on an uninfected bird, or which were examined before being allowed to feed on a bird at all! Hence, whatever their origin, there is no reason for associating them with Halteridium. For some cause or other, with the above-noted exception, these resting forms were never observed to become active, nor were active flagellates corresponding to them ever found.

In my permanent preparations, practically all these parasites occur in the resting condition (figs. 43–45): in one or two instances, however, there is an unmistakable wavy border in the anterior part of the body, accompanied by a drawn-out, tapering anterior end (figs. 46–47). I am strongly of the opinion that the flagellum is actually developed in both these cases, but it is difficult to be quite certain because for some reason or other, it has not stained red in the usual manner after Giemsa. Especially in the individual drawn in fig. 47, however, a definite line is evident along one edge of the anterior, tapering part, which is continued free for a short distance, which in all probability represents a flagellum. The general appearance of these forms, moreover, closely resembles that of the particular individual referred to above, just the instant before a well-marked, free flagellum became apparent. I think no one can have any doubt that these are really Flagellates, because they are certainly binucleate forms; in all cases, a definite kinetonucleus is present, usually immediately in front of the trophonucleus, which has the characteristic staining reaction to Giemsa. It may be pointed out that there is no question of this element being a karyosome, because, the karyosome of the nucleus can at times be seen, appearing, as is always the case in Giemsa-stained smears, as a clearer area, with a distinct centriole in the middle (cf. figs. 43 and 46). The cytoplasm always stains more darkly and much more bluish-purple in tint than any of the developmental phases of Trypanosoma noctuæ, in which, by the way, aflagellate phases appear to be entirely lacking. In all my experience of crithidial forms, whether as developmental phases of Trypanosomes, or "Crithidial" parasites, I have never observed any in which the cytoplasm stains in this peculiar dark manner, or in which the flagellum is so faint.

This parasite is certainly a crithidial form; this is evident from the contiguity of the two nuclei about the middle of the body, as well as from the distinct undulating membrane, where an individual is in, or about to assume, the active condition. In one respect it appears to be unique, i.e., in being non-flagellate, and moreover, without any trace of a rhizoplast, and quite motionless, when still possessing the elongated, fusiform shape, which is always associated in other Flagellates of this kind, with the active, flagellated condition; in all other cases of which I am aware, when the parasites are "resting" and non-flagellate they are in the short, more or less oval, haptomonad phase. This form does not agreė with any of the "Crithidiæ" hitherto described from mosquitoes; until more is known about it I am inclined to regard it as a distinct parasite. At present I have obtained the impression that this may be a purely Insectan form, a parasite more particularly, perhaps, of the larval Culex. Somehow, it does not look like a form accustomed to a blood-medium; its appearance and behaviour are so different from all the other crithidial forms which I have had occasion to study.

General Conclusion regarding the Question of a Connection between the Trypanosome and the Hæmosporidian Parasites of the Little Owl.

For the last time, I hope, that it will be necessary, I return to this subject, more particularly in order to point out the bearing upon it of the observations recorded above on the developmental phases of these parasites in the mosquito. It must be apparent, indeed, that these observations support and further strengthen the conclusion at which I had already arrived (14), that there is no connection whatever between these different types of parasite; and I have found nothing that in any way corroborates Mayer's account (2), in which he has upheld the opposite view.

In the first place, with regard to the mosquito which fed on Owl 19. As was to be expected from the typical ripe Halteridial infection of this bird, numerous fully-formed ookinetes were found without difficulty in the stomach when dissected. But not one of these ever showed any sign of passing into a flagellate condition; nor were any active Flagellates observed of the different types which I subsequently found frequently in mosquitoes fed on an owl known to have Trypanosomes. This fact is very significant when it is remembered that no Trypanosomes were ever seen in the blood of Owl 19, either in life or in searching smears, and if they were present, must have been so rare at the time as to be negligible.

As was discussed above, the matter was complicated just at the outset by the use of a few "wild" females in which a "resting Flagellate" occurred, which on one or two occasions developed into the active condition. In my own opinion, however, it is perfectly clear that these resting Flagellates have nothing to do with the ookinetes. Although there is undoubtedly a general resemblance in appearance between these two bodies when observed in life, there are several important reasons for concluding that this is only a coincidence. In stained preparations the two types of element appear fundamentally distinct, for the resting Flagellates show without exception the binucleate condition; the ookinetes, on the other hand, never do. In not a single instance, whether of Halteridium or of Leucocytozoon, have I been able to find an ookinete which possesses the binucleate condition, the first essential for it to be regarded as connected with a Hæmoflagellate. Again, the staining reaction of the general cytoplasm in the two cases is entirely different; and though, knowing what I do of the dangers, no less than the advantages, associated with the use of the Giemsa stain, I should be the last to lay stress upon casual staining differences in different cases; nevertheless, where such a difference is constant and uniform, weight may be laid upon it. Further, these resting Flagellates occurred also in "wild" females fed on owls totally uninfected with any of the parasites under discussion, and in which no ookinetes, of course, were found. Finally, these resting Flagellates have certainly nothing whatever to do with the developmental phases of Trypanosoma noctuæ.

Now, in the bred-out mosquitoes which fed on Owl 23, ookinetes were always scanty; particular individuals were carefully watched on different occasions, but in these also no change or development of any kind was ever seen. Nevertheless, in about 46 per cent. of these mosquitoes examined, active Flagellates were observed in different stages of development; and it is to be remembered that Owl 23 was the only bird in which Trypanosomes were found in the peripheral circulation, and actually at this period. If the Trypanosomes had indeed developed from ookinetes, it is difficult to understand why they should occur frequently when the ookinetes were scanty (and sometimes not actually noticed), and yet not at all in the case where the ookinetes were numerous (the female which fed on Owl 19, with the normal Halteridial infection). All my observations point clearly to these active Flagellates being the developmental forms of T. noctuæ in the mosquito, and derived directly from the stumpy, or stout fusiform Trypanosome, present in the blood in the summer (as previously described (**5**). I have never once seen the slightest indication of these developmental Trypanosome-stages in any mosquito fed on a bird which did not contain this stumpy, transmissive phase of the Trypanosome in the peripheral circulation—whether it contained Halteridium, or Leucocytozoon, or neither.

A few remarks, in concluding this discussion, about Mayer's account (l.c.). The most important statement of this worker is that, in hanging drops of blood from an owl infected with Halteridium, in which careful examination failed to reveal any Trypanosomes after four days or so Flagellates were found to be present; the inference is, of course, that these had developed from the Halteridium-ookinetes. I can only say that I feel absolutely convinced that Mayer was mistaken in supposing no Trypanosome-individuals to be present in the drop at the beginning of the experiment. I know well from experience how easily one of these forms can be overlooked, especially if the blood-corpuscles are in a fairly thick layer. Another possible explanation would be that certain very minute or ultramicroscopic phases of the Trypanosome were present, which later gave rise to flagellates. But this idea is quite unnecessary when I have shown clearly that the same particular flagellate developmental stages occur regularly, both in cultures and in mosquitoes, as a result of inoculation with a definite Avian Trypanosome form. For my own part, I say candidly that I can see no sufficient evidence up to the present for believing in "cryptotrypanosomiasis"[1] or in "infective granules," etc. In all the life-cycles of Trypanosomes which are now known in the invertebrate host, there is no suggestion of such a thing, and such an explanation is not, I consider, required. In birds, and even more so in cattle and sheep, Trypanosome individuals may be so excessively scanty in the circulation that the greatest difficulty is entailed in finding them; but let a single individual succeed in passing into a culture or into its right invertebrate host, and it will multiply so rapidly that before many days have passed the forms to which it gives rise are readily found. I may say also that I am no longer inclined to think that any small form of these Avian Trypanosomes occurs in the red blood-corpuscles, which might perhaps be mistaken for a young stage of Halteridium. Since very considerable doubt has been thrown upon the occurrence of such a stage in Trypanosoma cruzi, it is becoming more and more probable that Trypanosomes do not get into the red cells at all; at all events I am disinclined now to postulate the occurrence of such a phase in the Avian Trypanosomes which I have studied until we have an authentic instance of it in some other case. As in the case of fish-Trypanosomes, with which, as I have shown above, Avian Trypanosomes have much in common, I firmly believe that unless the transmissive trypanosome-form of an Avian Trypanosome passes into culture or the invertebrate host, no development of Flagellates will occur.

Mayer describes and figures further certain "large forms" which developed in mosquitoes, which he regards as the developmental stages of Leucocytozoon, in contradistinction to Halteridium. But these forms are obviously the same as certain of those which I have described and figured as the developmental stages of Trypanosoma noctuæ; his fig. 56, for instance, represents an individual nearly arrived at the final attenuated form. The stages which Mayer associates

[1] By this term I understand some definite phase of the parasite, hitherto unrecognised and possibly ultramicroscopic.

with Halteridium, equally with those which he associates with Leucocytozoon, constitute part of a regular series, and belong to a definite life-cycle, as I have clearly shown. Correspondingly, it will be remembered, Minchin and I showed also that the small forms of the Trypanosome in the blood, associated by Schaudinn with Halteridium noctuæ, form part of a regular series with the large individuals, regarded by him as belonging to Leucocytozoon ziemanni, and altogether represent only one species—Trypanosoma noctuæ; there is no species T. ziemanni. If, therefore, one still held to the idea that the small Trypanosomes are connected with Halteridium and the larger ones with Leucocytozoon, one would be led to the impossible position that Halteridium and Leucocytozoon are different phases of one and the same thing.

I do not suppose that Mayer any longer considers that an ontogenetic connection exists between any of these different parasites, especially as in addition to the above-mentioned paper, I have also published my account of the cytology, in which I have shown that, after all, there is no nuclear dimorphism in Halteridium and Leucocytozoon, and that these are not related to the Binucleata. But as Mayer's account is the only one published of late years on the developmental stages of these parasites of the owl in mosquitoes, I have been obliged to point out where it is erroneous in the light of my own observations on the whole subject.

The conclusion of the whole matter is, that the three parasites of the little owl—Trypanosoma noctuæ, Halteridium noctuæ and Leucocytozoon ziemanni—are entirely distinct and separate types; and the same is undoubtedly true for other species of these parasites in other birds. So far as T. noctuæ is concerned I have been able to outline above the main course of its development in the mosquito (Culex pipiens), though there are, unfortunately, gaps still to be filled up. The development of the Halteridium and the Leucocytozoon in the mosquito remains to be ascertained—supposing, that is to say, that there is any development beyond the ookinete stage. Other workers (e. g. the Sergents (**11**), Aragao (**1**) and Mayer (loc. cit.) have obtained the development of one or both of the parasites up to the same stage, but never any farther. As regards Halteridium, Aragao, who has succeeded in transmitting H. columbæ from a Hippoboscid fly (Lynchia) back again to the pigeon, is doubtful whether there is really any further development in the Insectan host; it is possible, moreover, that the so-called schizogony in the lung represents the delayed sporogony of the ookinetes, as has been hinted at by Minchin, in his text-book on the Protozoa (**4**). If that be so, then I see no reason why the ookinetes of H. noctuæ should not behave similarly; in which case, Culex pipiens may prove, after all, to be a true host—transmissive agent—of this parasite also. At any rate, for all that one can yet say to the contrary, the ookinetes may be inoculated back again into an owl, at the same time as the final propagative forms of the Trypanosome. It is, perhaps, not without significance that in every owl which was infected with the one parasite, we found the other also to be present (cf. Minchin and Woodcock, loc. cit.). The .development and transmission of Leucocytozoon are still more a matter of uncertainty. As far as Schaudinn's observations are concerned, if such a remarkable nuclear multiplication and skein-development does occur, it is very strange that neither Mayer nor I myself have seen any signs of it. One thing is, I think, practically certain; if the ookinete does produce a number of small elements by rapid division, these will not prove to be Trypanosomes or other binucleate Flagellates!

The Lister Institute;
May 12th, 1914.

References to Literature.

1. Aragao, H. de B.—"Ueber den Entwickelungsgang und die Uebertragung von Hæmoproteus columbæ," 'Arch. Protistenk.,' xii, 1908. p. 154, pls. 11–13.
2. Mayer, M.—"Ueber ein Halteridium und Leucocytozoon des Waldkauzes und deren Weiterentwickelung in Stechmücken," op. cit., xxi, 1911, p. 232, pls. 22 and 23.
3. Minchin, E. A.—"Observations on the Flagellates Parasitic in the Blood of Fresh-water Fishes," 'Proc. Zool. Soc.,' 1909, i, p. 2, pls. 1–5.
4. ——— 'An Introduction to the Study of the Protozoa.' London: Ed. Arnold, 1912.
5. ——— and Woodcock, H. M.—"Observations on the Trypanosome of the Little Owl (Athene noctua), etc.," 'Quart. Journ. Micr. Sci.,' 57, 1911, p. 141, pls. 20 and 21.
6. Reichenow, Ed.—"Die Hæmogregarinen," in 'Handb. d. path. Protozoen,' edited by Prowazek, S. v., vol. ii, Lief. 1, p. 602, 2 pls.
7. Robertson, M.—"Studies on a Trypanosome found in the Alimentary Canal of Pontobdella muricata," 'Proc. physic. Soc. Edin.,' 17, 1907, p. 83, pls. 4–7.
8. ——— "Further Notes on a Trypanosome found in Pontobdella muricata," 'Quart. Journ. Micr. Sci.,' 54, 1909, p. 119, pl. 9.
9. ——— "Transmission of Flagellates living in the Blood of certain Fresh-water Fishes," 'Trans. Roy. Soc.,' B. 202. 1911, p. 29, pls. 1 and 2.
10. Schaudinn, F.—"Generations- und Wirthswechsel bei Trypanosoma und Spirochæta," 'Arb. kais. Gesundhtsa.,' 20, 1904, p. 387. text-figs.
11. Sergent, E. and E.—"Études sur les Hématozoaires des Oiseaux," 'Ann. Inst. Pasteur,' 21, 1907, p. 251, pls. 6 and 7.
12. Woodcock, H. M.—"Studies on Avian Hæmoprotozoa: I. On certain Parasites of the Chaffinch (Fringilla cœlebs) and the Redpoll (Linota rufescens)," 'Quart. Journ. Micr. Sci.,' 55, 1910, 641, pls. 27–31.
13. ——— "On an Unusual Condition observed in Halteridium," 'Zool. Anz.,' 38, 1911, p. 465, text-figs.
14. ——— "Notes on Sporozoa, Nos. II–IV," 'Quart. Journ. Micr. Sci.,' 58, 1912, p. 171, pls. 9 and 10.

15. Woodcock, H. M.—"On 'Crithidia' fasciculata in Hibernating Mosquitoes (Culex pipiens), etc.," 'Zool. Anz.,' 43, 1914, p. 370, text-figs.

16. ——— "Further Remarks on the Flagellate Parasites of Culex, etc.," 'Op. cit., 44, 1914, p. 26.

EXPLANATION OF PLATES 29–31.

Illustrating Dr. H. M. Woodcock's paper on "Studies on Avian Hæmoprotozoa: No. III.—Observations on the Development of Trypanosoma noctuæ (of the Little Owl) in Culex pipiens; with Remarks on the Other Parasites occurring."

[All the figures on Pls. 29 and 30 are magnified 2000 times linear; those of the scheme, Pl. 31, are × 2000 (nearly). I am indebted to Miss Rhodes for kindly drawing and colouring most of the figures of the Halteridium ookinetes.]

PLATE 29.

All the figures relate to the development of T. noctuæ in the mosquito (Culex pipiens).

Figs. 1–3.—Typical trypanomonad forms.

Figs. 4–6.—Earliest stages in the development of the trypaniform type.

Figs. 7–10.—Intermediate trypaniform stages.

Figs. 11, 12.—Approximation to the final inoculative form. Fig. 11 shows an intermediate stage in the change in the nuclear condition; three fairly large chromatinic masses (karyosomes) are present.

Figs. 13–17.—Typical attenuated thread-like forms, with ladder-like nucleus. These are considered to be the inoculative type.

Figs. 18–21.—Individuals which agree in character with the final forms, except for the fact that the nucleus has not become ladder-like.

Figs. 22, 23.—Club-shaped trypanomonad forms. Early stages in the second line of development. (See text.)

Figs. 24, 26.—Pear-shaped forms, with the kinetonucleus tending to be in front of the trophonucleus and with hardly any membrane. These individuals result from the smaller member of an unequal division of